CHIROPTICAL PROPERTIES OF FLUORESCAMINE CONDENSATION COMPOUNDS WITH SECONDARY AMINO ACIDS IN SITU

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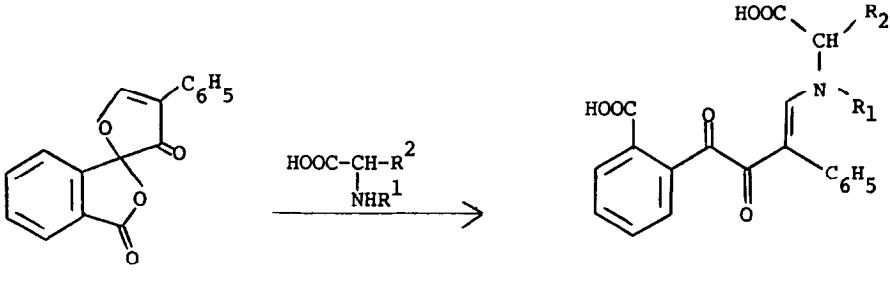
SUMMARY

Secondary amino acids react readily with fluorescamine to form aminoenone-type chromophores with long wavelength absorption maxima at 300-320 nm. The chiroptical properties of the reaction mixtures allow one to determine the absolute configuration of secondary amino acids in situ.

INTRODUCTION

We have recently described the application of fluorescamine, 4-phenylspiro [furan-2(3H), 1'-phthalan]-3,3'-dione I, as a chromophoric reagent for the determination of the absolute configuration of primary α-amino acids in situ (1). Fluorescamine also reacts directly with secondary amino acids (2,3) to form aminoenone-type chromophores II:



I II

The chromophore II is chiroptically active and CD spectra can be obtained from the reaction mixtures without isolation of the product. This reaction is simple and fast and can be performed in test tubes.

EXPERIMENTAL

A. Reagents

Fluorescamine (Fluram®) was obtained from Hoffmann-La Roche Inc. and histological grade dioxane from Fischer Scientific Co. N-Methyl-L-serine, N-methyl-L-threonine, N-methyl-L-phenylalanine, N-methyl-L-leucine and N-methyl-L-isoleucine were purchased from Bachem Inc. N-Methyl-L-valine, N-methyl-D-alanine, N-methyl-L-glutamic acid and L- and D-prolines were obtained from Fox Chemical Co. L-Hydroxyproline was purchased from Mann Research Laboratories and L-azetidine carboxylic acid from Pfaltz & Bauer Inc. D- and L-3,4-Dehydroprolines were provided by Dr. A. Felix, Hoffmann-La Roche Inc. The borate buffer (pH 9, 0.05 M) was prepared according to Clark and Lubs (4) using AR-grade chemicals from Mallinckrodt Chemical Works.

B. Method

Two ml of a 0.004 M solution of fluorescamine in dioxane are rapidly added to 2 ml of a 0.002 M (concentration may range between 10^{-2} and 0.5×10^{-6} M) solution of a secondary amino acid in 0.05 M borate buffer pH 9.0 in a test tube. The reaction mixture is stirred for 15 sec. on a Vortex-type mixer and transferred to a 0.1 cm cell (or into a cell of different length, depending on the amino acid concentration). After one minute the CD spectra were recorded on a JASCO Automatic Recording Spectropolarimeter, Model J-20 between 400 and 270 nm (between 400 and 220 nm for D- and L-prolines and 400 to 250 nm for N-methyl-L-leucine). The spectra are difficult to record below 270 nm because of the high absorption of the reagent, especially if its concentration is higher than 0.004 M or if longer cells are used.

RESULTS AND DISCUSSION

The optimum pH of the buffer was found to be 9.0. Under these conditions the reactions are complete within one minute at room temperature and the chromophore is stable at least for 2-4 hrs. When the amino acid concentration is low ($\leq 10^{-4}$ M) a 20-40 fold excess of the reagent is required (5), but in the concentration range of 0.01-0.001 M a twofold excess of fluorescamine is sufficient for analytical purposes.

The aminoenone-type chromophores arising from the reaction of fluorescamine or MDPF [2-methoxy-2,4-diphenyl-3(2H)-furanone] (6) with secondary amino acids are similar (2,3) and their absorption maxima are in the 250-255 (ε =ca 14500) and 300-320 nm regions (ε =16000-18000); an inflection is observed at 340-360 nm (ε =6000-10000).

The CD spectra of the reaction mixtures exhibit characteristic Cotton effects between 380 and 220 nm, but only those between 380 and 270 nm are readily

TABLE I

Cotton Effects in CD Spectra of Reaction Products of Secondary Amino Acids with Fluorescamine in Situ[a]

	1st		2nd		3rd Cotton Effect	
	λ nm	$[\theta] \times 10^{-3}$	λ nm	$[\theta] \times 10^{-3}$	λ nm	$[\theta] \times 10^{-3}$
N-Methyl-D-Alanine[c]	-	-	-	-	310	+ 4.20
N-Methyl-L-Leucine	356	- 1.61	325	- 2.45	297	-12.21
N-Methyl-L-Isoleucine	347	+ 1.92	-	-	307	- 6.57
N-Methyl-L-Valine[c]	-	-	-	-	303	- 7.38
N-Methyl-L-Threonine[c]	-	-	-	-	310	- 1.95
N-Methyl-L-Serine	345	- 2.51	-	-	305	- 7.70
N-Methyl-L-Glutamic Acid[b]	349	- 1.80	-	-	310	- 7.59
N-Methyl-L-Phenylalanine	340	- 8.19	313	+21.20	290	-24.05
L-Proline	348	- 1.11	320	+14.21	298	-18.11
D-Proline	350	+ 1.20	320	-14.55	299	+18.62
L-3,4-Dehydroproline	368	+ 1.21	321	+12.60	298	-19.00
D-3,4-Dehydroproline	366	-1.10	320	-13.60	299	+19.79
L-Hydroxyproline	369	+ 1.80	320	-10.40	299	-14.20
4-Allohydroxy-L-Proline	349	- 2.41	320	+15.98	297	-16.03
4-Allohydroxy-D-Proline	350	+ 3.00	319	-14.45	298	+15.50
L-Azetidine Carboxylic Acid	345	- 5.79	319	+ 8.35	295	-19.65

(a) With twofold excess of fluorescamine unless otherwise stated; (b) With twentyfold excess of fluorescamine;
(c) First and second Cotton effects are not observed under these experimental conditions.

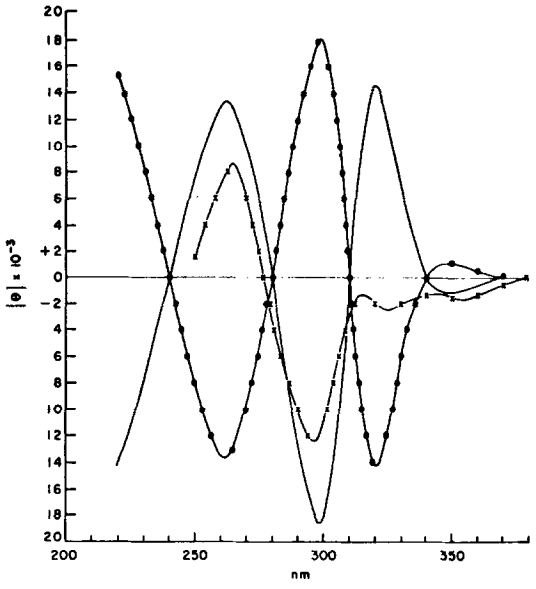


Fig. 1 CD spectra of the in situ reaction mixtures of L-proline (——), D-proline (●——●) and N-methyl-L-leucine (x——x) with fluorescamine in 0.05M borate buffer/dioxane, 1:1, v/v.

accessible. A number of secondary amino acids were reacted with fluorescamine and the CD spectra were recorded in the above-mentioned spectral range. The position and the intensity of the Cotton effects are summarized in Table I. In Fig. 1 the spectra of the D- and L-prolines and N-methyl-L-leucine derivatives are shown.

The strongest Cotton effects are observed in the 310-290 nm region (corresponding to the main UV maxima) and they are negative for chromophores derived from L-amino acids and positive in the case of D-amino acids. Within the experimental error, the CD curves of the L-amino acid-derived chromophores are mirror images of those of the corresponding D-amino acid derivatives.

Although the UV spectra of the reaction mixtures of various secondary amino acids with fluorescamine are quite similar (2,3), there are characteristic differences in the pattern of the CD curves between 380 and 320 nm which might be helpful for the early classification of an unknown secondary amino acid. The most striking difference is between the CD spectra of chromophores formed with cyclic and acyclic secondary amino acids, presumably due to differences in the conformational mobility.

Under standard conditions, as little as 0.1-1.0 μg/ml of secondary amino

acids have been routinely reacted with fluorescamine and useful CD spectra of the reaction mixtures were obtained.

As in the case of primary amino acids (1), the main advantage of this fluorescamine method is its simplicity. But for the determination of the absolute configuration of a more complex amino acid it may be essential to react the amino acid in question with MDPF and isolate and characterize the chromophoric derivative (7). This method also allows one to distinguish between secondary and primary amino acids because the chromophores formed with secondary amino acids have no Cotton effects in the 380 nm region.

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